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Review

Hydrolase-catalysed synthesis of peroxycarboxylic acids: Biocatalytic promiscuity for practical applications

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Abstract

The enzymatic promiscuity concept involves the possibility that one active site of an enzyme can catalyse several different chemical transformations. A rational understanding of the mechanistic reasons for this catalytic performance could lead to new practical applications. The capability of certain hydrolases to perform the perhydrolysis was described more than a decade ago, and recently its molecular basis has been elucidated. Remarkably, a similarity between perhydrolases (cofactor-free haloperoxidases) and serine hydrolases was found, with both groups of enzymes sharing a common catalytic triad, which suggests an evolution from a common ancestor. On the other hand, several biotechnological applications derived from the capability of hydrolases to catalyse the synthesis of peracids have been reported: the use of hydrolases as bleaching agents via in situ generation of peracids; (self)-epoxidation of unsaturated fatty acids, olefins, or plant oils, via Prileshajev epoxidation; Baeyer-Villiger reactions. In the present review, the molecular basis for this promiscuous hydrolase capability, as well as identified applications are reviewed and described in detail.

Keywords: Biocatalytic promiscuity; Hydrolases; Perhydrolysis; Bleaching; Epoxidation; Oxidation

Contents

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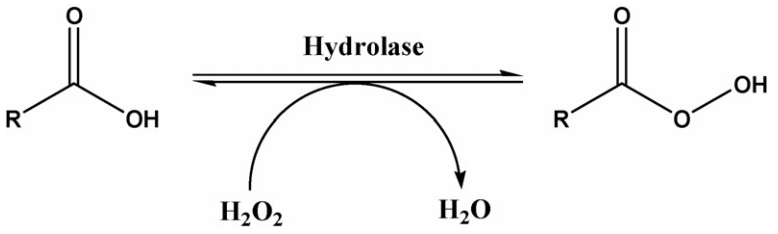
1. Introduction

Nowadays, one of the most outstanding concepts in biocatalysis is that one of the enzymatic promiscuity (O'Brien and Herschlag, 1999; Copley, 2003; Bornscheuer and Kazlauskas, 2004). This concept involves the ability of a certain enzyme (or group of enzymes) to catalyse different synthetic reactions, which can be more or less far from their natural role. In many of these examples the origin of such promiscuity can be found in the evolution of the enzymes from a common ancestor, which could have created/maintained different residual side-reactions (Babbit and Gerlt, 1997; Gerlt and Babbitt, 1998; Bugg, 2004). Obviously, this reveals an impressive potential of natural chemistry to adapt existing proteins to new biochemical pathways (Babbit and Gerlt, 1997). In an attempt to be systematic, catalytic promiscuity has been classified in four groups (Copley, 2003): (1) Use of analogue substrates by the same enzyme. (2) Reactions derived from the imperfect control of the non-natural reactants in the active site. (3) Totally different catalytic performances, using the same amino acidic residues of the enzymatic catalytic machinery. (4) New reactions derived from different amino acid residues within the active site. The importance of the promiscuity concept in biocatalysis is noteworthy, since a full and rational understanding of the causes could allow the development of new and useful enzymatic reactions by using "well-known" enzymes, as was recently reviewed (Bornscheuer and Kazlauskas, 2004).

Within biocatalysis, the use of hydrolases has been extensively documented. This group of enzymes – gathered in the α/β-hydrolase fold superfamily (Ollis et al., 1992; Perona and Craik, 1997; Bugg, 2004) – represents an excellent example of the enzymatic promiscuity and the applications of this phenomenon. Thus, despite that the natural role of the hydrolases is, among others, the cleavage of ester bonds of natural oils and/or short chain esters (lipases and esterases), as well as the hydrolysis of peptide bonds (proteases), their versatility has led to a wide number of applications in organic synthesis, making them the most widely used group of enzymes for practical purposes (de Zoete et al., 1994; Schmid and Verger, 1998; Bornscheuer and Kazlauskas, 1999; Hari Krishna and Karanth, 2002; Faber, 2004, and references therein). Notably, even reactions that are clearly different than the original ones have recently been published, such as hydrolase-catalysed C–C bond formation (Branneby et al., 2003) and the Michael addition synthesis (Cai et al., 2004; Torre et al., 2004; Carlqvist et al., 2005).

One of these non-conventional hydrolase-catalysed reactions is the perhydrolysis, that is, the use of H_2O_2 as



Scheme 1. Hydrolase-catalysed perhydrolysis (that is, use of H_2O_2 as nucleophile to form peracids catalysed by some hydrolases). The reversal reaction is also reported (Kirk et al., 1994).

nucleophile to form peracids catalysed by some hydrolases (Scheme 1).

This reaction was described more than a decade ago, and since then several applications have been reported (see below). In addition, the molecular basis of this "promiscuous" reaction has been elucidated recently (Bernhardt et al., 2005). Consequently a better understanding of the enzymatic performance has been achieved. This opens up the possibility to further exploit this promiscuity by development of tailor-made enzymes for specific applications.

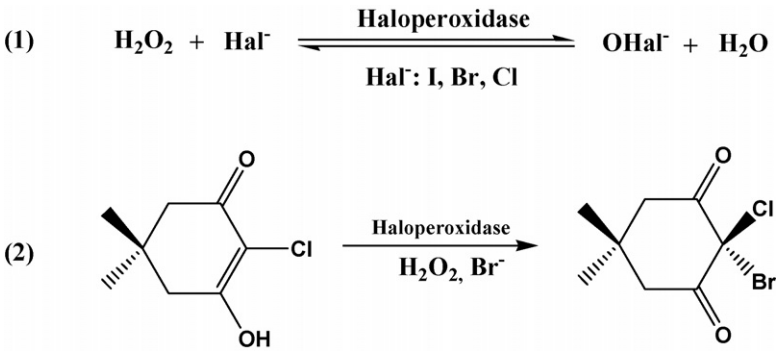
In the present paper, the molecular basis of the hydrolase-based perhydrolysis, as well as the current practical applications deriving from its promiscuous activity, are reviewed in detail.

2. Molecular basis for the perhydrolysis activity: hydrolases versus perhydrolases (cofactor-free haloperoxidases)

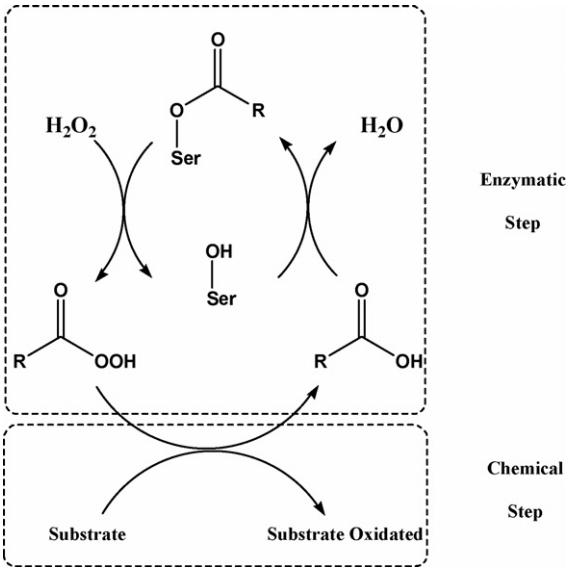
In 1985, Zacks and Klibanov showed that hydrolases working in organic media were able to accept nucleophiles different from water, for their catalytic performance. Thus, novel non-conventional reactions like, among others, aminolysis, thiotransesterification and oximolysis, were shown to be feasible (Zacks and Klibanov, 1985). This finding stimulated other groups to study whether the hydrogen peroxide could also be a nucleophile for hydrolases in organic media. This was confirmed several years later (Godtfredsen et al., 1991; Kirk et al., 1991). Hence, the concomitant development of sensitive analytic methods for detecting peracids in complex mixtures was crucial (Kunath et al., 1991; Kirk et al., 1992).

In addition to hydrolases, it is well known that other enzymes, namely haloperoxidases, are able to use H_2O_2 as substrate. Those enzymes are named after the most electronegative halide that they are able to oxidize, and are classified according to their cofactor dependence as heme-type, vanadium-dependent and metal-free haloperoxidases (Hofmann et al., 1998). Haloperoxidases catalyse the formation of hypohalites from hydrogen peroxide and chloride, bromide, or iodide. Later on, the electrophiles thus formed are able to halogenate suitable organic substrates, as depicted in Scheme 2 (Picard et al., 1997).

Amongst haloperoxidases, the so-called non-heme-, metal-free-, or cofactor-free-haloperoxidases appeared to be remarkably interesting (van Pee et al., 1987; Wiessner et al., 1988; Pelletier and Altenbuchner, 1995; Hofmann et al., 1998; Kataoka et al., 2000, and references therein), since a catalytic triad Ser-Asp-His – analogous to that one reported for serine-hydrolases – was also located within the active site of these biocatalysts (Pelletier et al., 1995). Furthermore, it was observed that not only certain hydrolases could show some perhydrolase activity, and even act as haloperoxidases (Kirk and Conrad, 1999; Cheeseman et al., 2004), but also some of those cofactor-free haloperoxidases are able to perform hydrolytic reactions in a residual extent (Pelletier et al., 1995; Pelletier and Altenbuchner, 1995; Picard et al., 1997; Cheeseman et al., 2004). Consequently, it was speculated whether both groups of enzymes shared a common origin and an analogous catalytic mechanism,



Scheme 2. Natural role of haloperoxidases (for more information see Picard et al., 1997; Hofmann et al., 1998, and references therein).



Scheme 3. Postulated common mechanism for hydrolases and perhydrolases (Picard et al., 1997).

in which the involvement of the catalytic triad would be crucial (Pelletier et al., 1995). Subsequent studies confirmed this assumption (Picard et al., 1997; Kirk and Conrad, 1999; Bernhardt et al., 2005). Thus in both groups of enzymes the catalytic performance enables the enzymatic formation of a peracid in a first step, while in a later stage this molecule is thought to be the responsible for the further oxidation observed, through a chemical mechanism which was proposed many years ago (Swern, 1946). This two-step chemoenzymatic reaction hypothesis was supported by the fact that the haloperoxidases strictly require the presence of acetate ions for their catalytical performance, which suggests the existence of the peracid as intermediate (peracetic acid) (Hecht et al., 1994; Pelletier et al., 1995; Picard et al., 1997; Hofmann et al., 1998; Kirk and Conrad, 1999). In addition, the total absence of enantioselectivity observed in the second step (oxidation process) also seems to support the assumption that a chemical mechanism is involved in the second step (Picard et al., 1997; Faber, 2004) (Scheme 3).

On the other hand, an aryl-esterase from *Pseudomonas fluorescens* has attracted enormous attention (Choi et al., 1990; Pelletier and Altenbuchner, 1995; Pelletier et al., 1995; Hofmann et al., 1998), as its structure seems to be the "missing-link" between haloperoxidases and hydrolases (Cheeseman et al., 2004; Bernhardt et al., 2005). Actually, from such structural and mechanistical analogies, some authors have argued whether both reaction performances (one for serine-hydrolases and one for perhydrolases) are really different or are part of a common mechanism of enzymatic catalysis (Kirk and Conrad, 1999).

However, the explanation why, if both kinds of enzymes showed the same similar structure and mechanism, the esterases displayed higher esterase activity, while haloperoxidases enabled higher haloperoxidase activity was still unknown. Furthermore, the existence of some hydrolases (i.e., subtilisin) with a catalytic triad that does not display any perhydrolase activity is also common knowledge (Bernhardt et al., 2005). An explanation proposed for the differences is that the electronegative microenvironment of the active site in case of haloperoxidases could be responsible for this difference in selectivity, since the more hydrophobic environment present in haloperoxidases – when compared to other hydrolases – would protect the peracid against hydrolysis while halide is transported to the active site (Hofmann et al., 1998). Besides, it was also suggested that other aminoacidic residues, different from the catalytic ones, could play a role in the enzymatic performance (Hofmann et al., 1998). This difference in activities between hydrolases and haloperoxidases could also be partially related to the presence, in the structure of the enzyme, of amino acids particularly sensitive to oxidation by H_2O_2/peracids (Pelletier et al., 1995). In this respect, recent studies on *Candida antarctica* lipase B revealed that the residue Met72 – located closely to the active site – could be one of those responsible groups, as mutations on this position led to more effective catalysts in the perhydrolysis reaction (Patkar et al., 1998; for recent articles on CAL-B mutations, see Zhang et al., 2003; Lutz, 2004; Chodorge et al., 2005). However, those facts do not seem to be sufficient to fully elucidate the differences observed.

An interesting explanation has been recently proposed. In a first study, the crystallographic structure of the *P. fluorescens* aryl-esterase did not show any remarkable difference from that for homologous perhydrolase (Cheeseman et al., 2004). Subsequently, the alignment of the amino acid sequences of six hydrolases and six perhydrolases was performed, to observe which residues appeared in the perhydrolases, but not

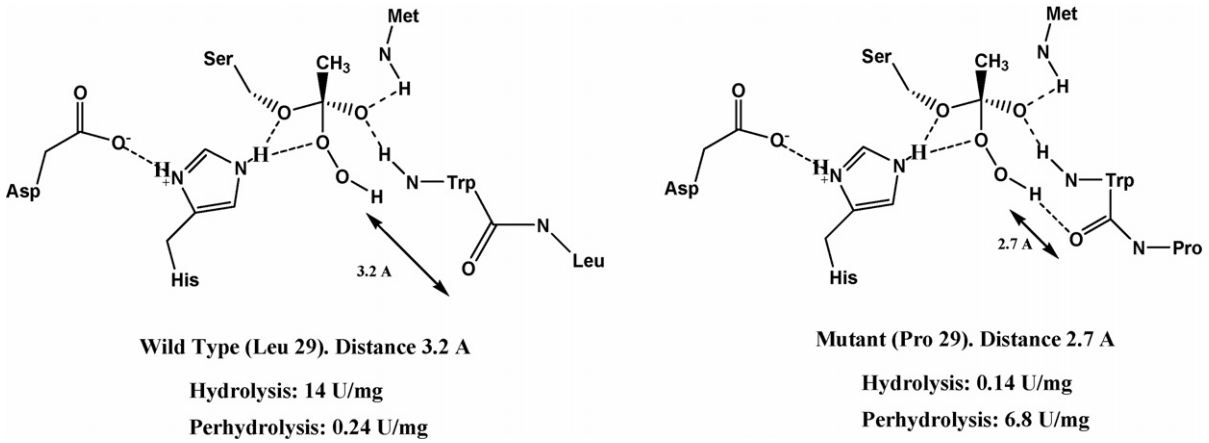


Fig. 1. Proposed molecular basis of perhydrolase activity in *P. fluorescens* aryl-esterase, according to data recently published. In the wild type (left) the (long) distance between Leu29 and hydrogen peroxide avoids the stabilisation of the substrate. In the mutant form (right, with Pro29), the substrate (H_2O_2) is stabilised by a key hydrogen bond. Data and figure taken from Bernhardt et al. (2005).

in the esterases (Bernhardt et al., 2005). Those residues within a sphere of 12 Å around the reactive –OH of the serine of the catalytic triad were mutated by molecular biology techniques. Notably, the substitution of the Leu29 with a Pro led to an increase of the perhydrolase activity, compared to that displayed by the wild-type catalyst, and additionally to a decreased esterase activity of the catalyst. Through molecular modelling it was reported that the substitution of that amino acid led to a change in the distance between the catalytic serine and the carbonylic group of the Pro29, that is, 3.2 Å in the wild-type enzyme (leu), and 2.7 Å in the mutated catalyst (Pro). This shorter distance enabled the formation of a hydrogen bond between the peroxide nucleophile and the new proline group, which stabilised the hydrogen peroxide–active site interaction, and facilitated the perhydrolysis reaction (Bernhardt et al., 2005). This new and impressive finding opens up the possibility of enhancing the catalytic promiscuity of *old* enzymes to perform completely new reactions. A graphical explanation of the statement is depicted in Fig. 1.

3. Applications of the (per)hydrolase-catalysed synthesis of peracids for bleaching

The use of hydrogen peroxide as bleaching agent – because of its oxidising power – is well known since a long time. Due to its instability and problematic handling, it was produced in situ by using sodium perborate and high temperature (Hofmann et al., 1992). Thus, in the 1950s peroxyacids and peroxyacid-generating bleaches were recognised for their potential to provide efficient bleaching at lower washing temperatures (Grime and Clauss, 1990), and patents regarding the use of mixtures of these chemicals were filed (Bossu and Kacher, 1987, and references therein). Especially medium chain alkyl peracids turned out to be very effective for the bleaching system (Estell, 1993). These molecules have found interesting applications in, i.e., the cotton fabric (Rucker and Cates, 1988; Kirk et al., 1994). However, neither hydrogen peroxide, nor the organic peracids are stable. Therefore, an in situ generation is almost mandatory for an effective bleaching procedure.

It was evaluated whether the enzymes could be used to form the peracids for bleaching compositions, thus enhancing the feasibility of the process. The combination of an ester-hydrolysing enzyme, an acyl-alkyl ester, and a percompound, turned out to be a useful bleaching agent. This process allowed bleaching at lower temperatures, around 25–80 °C. It was assumed that the enzyme hydrolysed the ester to yield the peracid (Weyn, 1976). Since the process proceeds in aqueous media, a close interaction between the peracid, the H_2O_2, and the hydrolase, is expected. In such conditions the catalyst is highly sensitive to denaturation, and consequently, it is important to find a

biocatalyst able to work in that environment. In this respect, a patent in which a novel lipase from *Pseudomonas putida* appeared to be useful for such purposes, being resistant to H_2O_2, was filed (Wiersema and Stanislowski, 1987; Wiersema et al., 1987, 1991). In all examples of this patent, a triglyceride (i.e. trioctanoin) was used as the acyl source for the production of the organic peracid. This result was later confirmed by another group, although less clearly (Kirk et al., 1994). Within the same area, a patent concerning the use of proteases (i.e. esperase®, alcalase®, carboxypeptidase A or α-chymotrypsin) as biocatalysts for the production of peracids in bleaching, was also granted (Stanislowski et al., 1994). However, contrasting results have been reported in literature when the same experiments involving proteases were performed (Kirk et al., 1994).

As mentioned before, the bleaching action takes place in aqueous environment. This aspect leads to a nucleophilic competition between the H_2O/H_2O_2 for the acyl–enzyme complex of the hydrolase. Thus, the ratio of perhydrolysis-rate to hydrolysis-rate (P/H) in a reaction mixture is crucial to determine the efficiency of the enzymatic bleaching (Estell, 1993). Yet, there is still a lack of systematic in the determination of the perhydrolysis/hydrolysis parameter, since depending on the application different substrates/strategies have been employed (Poulose, 1994). The method for the evaluation involves usually an ester (commonly aliphatic derivatives), water, and H_2O_2, but more fixed criteria for the measurement would be necessary to accurately compare results from several laboratories. This fact could be an interesting future academic task (for different P/H measurements see Wiersema et al., 1991; Poulose, 1994; Amin et al., 2005).

On the other hand, it was stated that hydrolases were also capable to perform the reversal reaction, and form hydrogen peroxide and carboxylic acids from the parent peracid in aqueous solutions (Kirk et al., 1994). Related to this, most of the commercial hydrolases in aqueous media show a P/H value not suitable for practical applications. Thus, attempts to improve this were conducted by using molecular biology techniques. A lipase from *Pseudomonas putida* that showed a P/H value of 0.25 was discovered (Estell, 1993). Subsequently, mutations on several positions close to the active site were done. When the Gln127 was replaced by Ser, and Ser205 was mutated into Thr, the hydrolysis rate dropped dramatically, whereas the perhydrolysis rate only decreased slightly. In this case, a P/H of 1 was achieved, which made the process competitive for bleaching applications (Poulose and Anderson, 1992; Estell, 1993). These findings, developed more than a decade ago, seem to be interestingly familiar to those recently reported by Bernhardt et al., in which the molecular basis for hydrolysis versus perhydrolysis was clarified (see Section 2) (Bernhardt et al., 2005).

Finally, a patent for bleaching process has been recently claimed (Amin et al., 2005). In this invention, a perhydrolase from *Mycobacterium smegmatis* is used for generating peracids, and a P/H value higher than that one observed for *common* hydrolases is reported for this type of enzymes. Specifically, a P/H value greater than 1 was stated. Furthermore, by working on mutants this value was even increased up to 5 (Amin et al., 2005). Notably, other sources of H_2O_2 can be used, for example, an enzymatic coupled system in which glucose oxidase is employed to generate the hydrogen peroxide. In a first step glucose is converted (catalysed by glucose oxidase) to gluconic acid, with production of H_2O_2, which is subsequently used by the perhydrolase (Schemes 1 and 3). Moreover, according to the patent, other different oxidases are also useful for the proposed process (Amin et al., 2005).

4. The hydrolase-catalysed "oxidation": synthetic applications

4.1. Perhydrolysis in organic media: synthesis of peracids

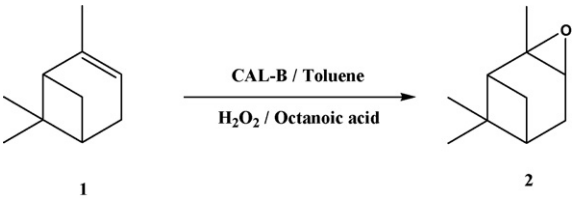
The hydrolase-catalysed perhydrolysis proceeds better in organic solvent, as a lower nucleophilic competition (H_2O/H_2O_2) is expected in these media. In addition, the equilibrium is shifted towards the synthesis (Kirk et al., 1994). Therefore, the hydrolase-catalysed synthesis of peracids is feasible in these media with high conversions. For such purpose, the lipase B from *Candida antarctica*, immobilised on polyacrylate resin, working in toluene, gave the best results (Yadav and Devi, 2002, and references therein). This lipase is one of the most utilised enzymes within the hydrolase field, with an impressive versatility, including this efficient perhydrolytical activity (for recent reviews on CAL-B, see Anderson et al., 1998; Nielsen et al., 1999; Kirk and Christensen, 2002).

Recently, the enzymatic synthesis of perlauric acid was studied in toluene (Yadav and Devi, 2002). This solvent was also chosen by other authors as the best one for such purposes (Björkling et al., 1990, 1992; Godtfredsen et al., 1991). The empirical data were adjusted to a ping–pong Bi–Bi mechanism (Yadav and Devi, 2002; for the definition and explanation of the ping–pong Bi–Bi mechanism, see Segel, 1975; Paiva et al., 2000, and references therein).

On the other hand, the hydrogen peroxide concentration turned out to be crucial for the efficiency of the process. When its concentration was enhanced, better conversions were achieved, but at extremely high concentration the denaturation of the enzyme occurred (Yadav and Devi, 2002). This deactivation, further studied by these authors, showed a pseudo first order kinetics. Furthermore, it was observed that at lower temperature the rate of the reaction was faster than the rate of deactivation, suggesting that, if the H_2O_2 concentration could be lowered in the vicinity of the active site of the enzyme, the deactivation would be suppressed, and thus its reusability possible (Yadav and Devi, 2002). The feasibility of this approach for this reaction has been demonstrated by studies in a hollow-fibre membrane reactor, showing an enhancement of the enzyme stability (Cuperus et al., 1994; Kramer et al., 1994).

4.2. Prileshajev oxidation

The in situ formed peracid has been used for the Prileshajev oxidation of double bonds, in very mild conditions. For such purpose, two-phase systems, solvent-free reactions, and organic solvents have been successfully tested (Björkling et al., 1990, 1992; Miura and Yamane, 1997; Klaas and Warwel, 1999; Skouridou et al., 2003a,b). Since the enzyme is not resistant to high concentrations of hydrogen peroxide, its gradual addition improves the process by reducing/slowing the deactivation of the biocatalyst (Björkling et al., 1990, 1992; Godtfredsen et al., 1991). In this respect, the immobilisation of the enzymes in photo-crosslinkable polymers enhanced its synthetical capability, as a protection matrix was formed (Miura and Yamane, 1997). Thus, it was possible to demonstrate that *C. rugosa* lipase (formerly *C. cylindracea*) was a good catalyst in such immobilised forms, while the free enzyme did not display any perhydrolytical activity (Björkling et al., 1992; Miura and Yamane, 1997). Cyclohexene and 1-octene have usually been selected as substrates to evaluate the feasibility of the reaction.

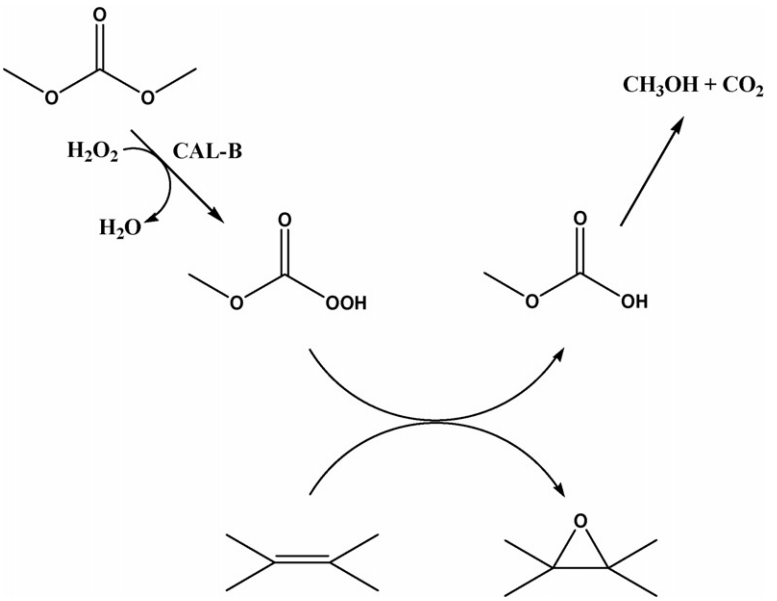


Scheme 4. Lipase-mediated Prileshajev epoxidation of α-pinene reported (Skouridou et al., 2003a,b).

In addition, during the last years the Prileshajev epoxidation has also been used in the chemistry of pinenes, since the epoxided forms of these molecules (as well as other monoterpenes), are very useful intermediates and chiral building blocks (Gusevskaya et al., 1998). Thus, the α-pinene **1** was successfully epoxidated enzymatically by using *C. antarctica* lipase B, octanoic acid and hydrogen peroxide (Scheme 4) (Skouridou et al., 2003a,b). Also in this case, a gradual addition of the hydrogen peroxide enhanced the epoxide production, allowing the enzyme to act efficiently after several re-uses, which enhances the practical feasibility of the reaction. Furthermore, a complete study of the variables that control the synthesis was recently carried out (Skouridou et al., 2003a,b).

Klaas and Warwel have reported an alternative, in which the Prileshajev epoxidation proceeds in "acid-free" conditions (Klaas and Warwel, 1999). The new approach is especially interesting for those substrates, which show acid-sensitivity, like the β-pinene. Hence, instead of a carboxylic acid, dimethyl carbonate was used, and so, after the epoxidation step, the monomethyl ester will probably decompose to methanol and carbon dioxide (Scheme 5) (Klaas and Warwel, 1999).

The Prileshajev epoxidation has also found application in the field of unsaturated fatty acids and oils. It was demonstrated that, when unsaturated fatty acids were used as substrates, a "self"-epoxidation proceeded (Scheme 6). According to the studies, a competition between inter- and intra-molecular epoxidation was observed. In this case, a 15-fold re-use of the enzyme (CAL-B, Nov-435) was reported, which makes the process competitive from an economic point of view



Scheme 5. Lipase-mediated Prileshajev epoxidation: acid-free approach (Klaas and Warwel, 1999).

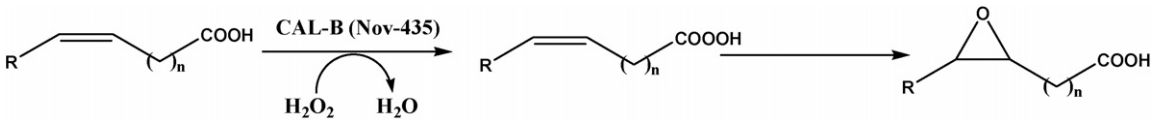
(Warwel and Klaas, 1995). More recently, efficient epoxidation of oleic acid with Nov-435 in solvent-free system has been reported, as well as new analytic techniques for this reaction (Orellana-Coca et al., 2005a,b).

The same reaction was successfully performed when unsaturated fatty esters were used (Klaas and Warwel, 1996, 1997). This is important, since the higher reactivity of the ester, compared to that of the acid, allows the application of hydrogen peroxide at 35% (v/v), instead of 60% (v/v), as required during the use of the free acids. Actually, the handling of 35% (v/v) hydrogen peroxide offers a safer environment and this solution is a standard commercially available chemical (Klaas and Warwel, 1997).

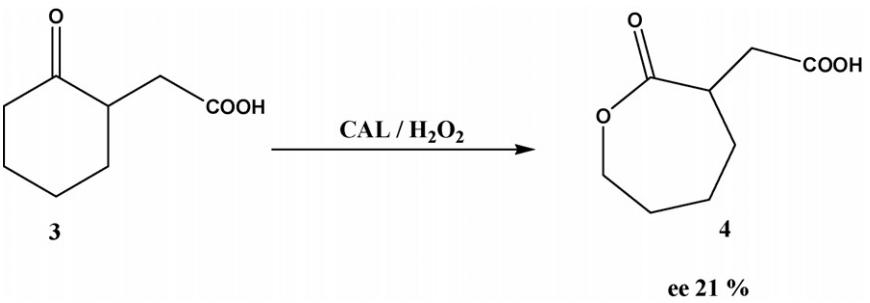
A similar approach has been reported for the mild epoxidation of plant oils (Klaas and Warwel, 1996; Hilker et al., 2001). A mixture of tri-, di-, and mono-epoxidated glycerides as well as epoxidated fatty acids were obtained at the end of the reaction when the plant oil was added. This is due to the absence of acids at the beginning of the reaction, which leads to a peracid formation at the cost of the partial hydrolysis of the triglycerides. To overcome this problematic mixture, the reaction was performed with an initial addition of 5% of free fatty acids, which were actually the enzyme substrate to form the peracids. As a further separation of those free fatty acids is simple, different epoxidated plant oils (i.e., rapeseed, sunflower, or linseed oil) were isolated in good yields (Klaas and Warwel, 1996; Schmid and Verger, 1998; Biermann et al., 2000; Hilker et al., 2001). In addition, a mathematical model for this reaction was developed and successfully applied (Hilker et al., 2001).

4.3. Baeyer-Villiger oxidation

Another interesting application of the hydrolase-catalysed synthesis of peracids is the Baeyer-Villiger



Scheme 6. Self-epoxidation of unsaturated fatty acids via lipase-mediated formation of peracids.



Scheme 7. Lipase-mediated Baeyer-Villiger reaction, in which some enantioselection was reported. This is supposed to occur because of the intramolecular reaction of a chiral peracid (Lemoult et al., 1995).

reaction. In this area, one paper describes the capability of the reaction mixture (myristic acid + *C. antarctica* lipase + hydrogen peroxide) to yield lactones starting from cyclic ketones (Lemoult et al., 1995). Remarkably, one of the lactones (**4**) was obtained in a modest optically active form (21% enantiomeric excess) (Scheme 7).

The existence of enantioselectivity does not fit with the proposed mechanism, in which the oxidation proceeds without the participation of the enzyme (Scheme 2) (Picard et al., 1997; Faber, 2004). It was suggested that the enzyme formed the peracid more rapidly by one enantiomer, and thus, the intramolecular Baeyer-Villiger reaction would lead to the slight enantioselectivity observed (Lemoult et al., 1995). Although the existence of "real" enzymatic Baeyer-Villiger oxidations is well and widely reported in the open literature (as recent examples, see Carballeira et al., 2004; Bocola et al., 2005; Moonen et al., 2005; Ottolina et al., 2005; Sicard et al., 2005; Gutiérrez et al., 2005, and references therein), the lipase-mediated approach could represent an easier alternative for practical purposes.

4.4. Other applications

The lipase-mediate approach for generating peroxycarboxylic acids in situ has been applied for the S-oxidation of penicillin G. Although this process proceeded also without enzyme, its catalysis enhanced the efficiency (de Zoete et al., 1993). In the case of penicillin V the selective oxidation has been reported to be complete (Kirk, 1991). On the other hand, it should be stressed that enzymatic approach allows to overcome the handling problems of the isolated peroxycarboxilic acid. Theoretically, any peracid-mediated oxidation would be feasible with the enzymatic approach (for applications of peracids as oxidative agents, see Augustine, 1969). Moreover, application of the epoxidation reactions in ionic liquids has been also reported as successful (Madeira Lau et al., 2000).

Finally, the lipase-based preparation of optically active chiral hydroperoxides, as potential stereoselective oxidizing reagents, has been described. The lipase has been employed for the kinetic resolution of hydroperoxides in organic solvents by using analogous procedure to conventional kinetic resolution of alcohols or other racemates (Baba et al., 1988; Höft et al., 1995), yielding hydroperoxides in high enantiomeric excess.

5. Conclusions and outlook

The topic reported in this review is an example of biocatalytic promiscuity, in which, by exploiting a non-natural activity of hydrolases, a substantial number of practical applications can be achieved. In addition, the recent understanding of the molecular basis of this catalytic performance opens up the possibility for new applications in the near future, where tailor-made (per)hydrolases might be obtained by molecular biology techniques. This strategy could lead, for instance, to biocatalysts more resistant to the drastic reaction conditions originating from the use of hydrogen peroxide and peracids at high concentrations. Furthermore, an enhancement of the perhydrolysis/hydrolysis rate ratio (P/H), which could improve the economical

impact of the new technology, is highly feasible with this approach, as some examples described above have shown.

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